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Research Article

DEVELOPMENT AND VALIDATION OF ABSORBANCE CORRECTION METHOD FOR SIMULTANEOUS ESTIMATION OF ALISKIREN AND AMLODIPINE IN COMBINED DOSAGE FORM

PATEL SAMIXA RAMESHBHAI.*[1], PATEL CHHAGANBHAI NANJIBHAI. [2]

[1]School of Pharmacy, RK University, Rajkot, India, Atmiya Institute of Pharmacy, Department of Pharmaceutical Chemistry,"Yogidham Gurukul", Kalawad road, Rajkot-360005, Gujarat, India.

[2] Shri Sarvajanik Pharmacy College**,** Mehsana- 384001, Gujarat, India.

* Author for correspondence

Email: samixa.patel@gmail.com

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ABSTRACT: The present paper describes simple, accurate, rapid, precise and sensitive UV spectrophotometric absorption correction method for the simultaneous determination of amlodipine and aliskiren in combined tablet dosage form. methanol was used as solvent. The wavelengths selected for the analysis using absorption correction method were 354.5 nm and 256.0 nm for estimation of amlodipine and aliskiren respectively. Beer's law obeyed in the concentration range of 10-60 µg/mL and 20-120 µg/mL for amlodipine and aliskiren, respectively. The mean percentage drug content for amlodipine and aliskiren were found to be 99.9. ± 1.38 and 99.87 ± 1.25 respectively and the % RSD value was found to be less than 2 which shows the precision of method. The high recovery and low coefficients of variation conforms the suitability for the routine quality control analysis of amlodipine and aliskiren in pure and pharmaceutical dosage forms.

Key words: Amlodipine, Aliskiren, Spectrophotometric absorption correction method

INTRODUCTION

Aliskiren(2*S*,4*S*,5*S*,7*S*)-5-amino-*N*-(2-carbamoyl-2,2-dimethylethyl)-4-hydroxy-7-{[4-methoxy-3-(3 methoxypropoxy) phenyl]methyl}-8-methyl-2-(propan-2-yl) nonanamide (Fig.1) is rennin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotens in II [1]. Literature survey revealed HPLC[2], RP-HPLC[3-5], simultaneous UVspectrophotometric [6] and spectroflurimetric[7] methods are reported for the estimation of aliskiren hemifumarate alone or in combination with other anti-hypertensive agents. Amlodipine ((RS)-3-ethyl 5-methyl 2-[(2- aminoethoxy) methyl]-4-(2-chlorophenyl)-6- methyl- 1, 4-dihydropyridine-3,5-dicarboxylate)[8] is long acting calcium channel blockers used as an antihypertensive and in the treatment of angina. It acts by relaxing the smooth muscle in the atrial wall, decreasing total peripheral resistance and hence reducing blood pressure, in angina it increases blood flow to the heart muscle. Various analytical methods have been reported for the assay of Amlodipine in pure form as well as in pharmaceutical formulation. They include HPLC [9, 10], HPTLC [11], RP-HPLC [12, 13], Gas chromatography [14], mass-spectrometry [15], and flourimetry[16]. Aliskiren and Amlodipine combination is not official in any pharmacopeias so any official analytical method is not available for estimation of these two drugs. One spectroscopy method is available for estimation of Aliskiren and Amlodipine in tablet dosage form [17]. But no any absorbance correction method is available for determination of Aliskiren and Amlodipine in combine dosage forms. So aim of the present study was to develop accurate, precise and selective absorbance correction method assay procedure for the analysis of Aliskiren and Amlodipine in combined dosage forms.

EXPERIMENTAL

Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Sartorius CP224S analytical balance (shimadzu, Japan),Ultra sonic cleaner (Life care eq. PVT. LTD, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study.

Reagents and Materials

Pharmaceutical grade of Aliskiren (ALK) as a gift samples from Novartis India limited, India and Amlodipine besylate were kindly supplied as a gift samples from Torrent research centre, Gujarat (India),The pharmaceutical formulation (Tekamlo™) containing 150 mg ALK and 5 mg AML was procured from the local pharmacy, AR grade Methanol (S.D. Fine Chemical Ltd., Mumbai, India), Whatman filter paper no. 41 (Whatman International Ltd., England). **Preparation of ALK and AML Standard Solutions**

A mixed stock solution of ALK (2000 µg/Ml) and AML (1000 µg/Ml was prepared by accurately weighing ALK (200 mg) and Amlodipine besylate (138 mg) which is equivalent to AML (100 mg), dissolving in methanol and diluted to 100 Ml with methanol in the same volumametric flask

Preparation of Sample Solutions

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 150 mg of ALK and 5 mg of AML was transferred to a 100 Ml volumetric flask. The content was mixed with methanol (60 Ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtered through a Whatman filter paper no. 41. The volume was adjusted up to the mark with methanol. An aliquot of this solution (0.6 Ml) was transferred in to a 10 Ml volumetric flask and Addition of 2 Ml AML standard solution (100µg/Ml) to the same volumetric flask. Methanol was transferred to this volumetric flask and Volume was made up to the mark to give a solution containing 90 µg/Ml ALK and 23µg/Ml AML. This solution was used for the estimation of ALK and AML.

Determination of the analytical wavelengths

Absorbance spectrum of pure AML was scanned in the spectrum basic mode. Using the cursor function, the absorbance corresponding to 354.5 nm (wavelength λ_1 for AML) was noted from spectrum. Then the cursor function was moved along with peak curve until the absorbance equal to that of absorbance at 354.5 nm was found. The wavelength obtain corresponding to this absorbance value was 256 nm (wavelength λ_2). The absorbance of various dilutions of AML in methanol was measured at 354.5 nm. Absorbance spectrum of pure ALK was also scanned in the spectrum basic mode. ALK showed some absorbance value at 256.0 nm (λ_2) while it dose not show any absorbance value at 354.5 nm. The absorbance value at 354.5 nm is due to AML only in the combined mixture of both drugs. Wavelength λ_1 (354.5 nm) was selected for the measurement of AML.

METHOD VALIDATION:

Calibration curve (Linearity)

Calibration curves were plotted over a concentration range of 20-120 µg/Ml for ALK and 10-60 µg/Ml for AML. Accurately measured mixed standard working solutions of ALK and AML (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 Ml) were transferred to a series of 10 Ml of volumetric flasks and diluted to the mark with methanol and absorbance's were measured at 354.5 nm and 256 nm for both the drugs. The calibration curves were constructed by plotting absorbance at 354.5 nm versus concentrations for AML and absorbance difference (A_{256} - $A_{354.5}$) vs concentration for ALK.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of ALK and AML by the standard addition method. Standard solutions of ALK (20, 40, 60 µg/Ml) for and AML (10, 20, 30 µg/Ml) were added to prequantified sample solutions of ALK (40 µg/Ml) and AML (20 µg/Ml) tablet dosage form. The amounts of ALK and AML were estimated by applying obtained values to the regression equation of the calibration curve.

Method Precision (% Repeatability)

The precision of the instruments was checked by repeated scanning and measurement of absorbance of solution of (n = 6) of ALK and AML (40 µg/Ml) without changing the parameter.

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of ALK (40, 80, and 120 µg/Ml) and AML (20, 40, and 60 µg/Ml). The results were reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

LOD and LOQ of the drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guideline:

LOD = 3.3 × □/S

LOQ = 10 × □S

Where □= the standard deviation of the response

S = Slope of calibration curve.

ANALYSIS OF ALK AND AML IN COMBINED DOSAGE FORM:-

The response of formulations was measured at 354.5nm and 256 nm for AML and ALK, respectively by proposed method as described above. The amounts of ALK and AML present in sample solution were determined by fitting the responses into the regression equation of calibration curve of the proposed method.

RESULTS AND DISCUSSION

Method development

The utility of dual wavelength data processing program is its ability to calculate unknown concentration of component of interest in a mixture containing an interfering component. For elimination of the effects of an interfering component, two specific wavelengths are chosen. First wavelength λ_1 at which minimum absorbance of AML was observed and there was no interference of ALK at this wavelength (354.5nm). Second wavelength λ_2 was the wavelengths at which the absorbance of AML was same as at λ_1, and also ALK was also give some absorbance at this wavelength (256.0 nm). In this proposed method the absorbance of ALK alone in a mixture of ALK and AML was determined using dual wavelength data processing program. To remove the interference of AML to the absorbance at 256.0 nm (λ_2), the wavelength of minimum absorbance for AML, another wavelength 354.5nm (λ_1) was found out at which the absorbance of ALK was zero. This was confirmed by measuring the absorbance of various dilution of AML in methanol at 256.0 nm and 354.5nm. The absorbance at these two wavelengths was found to be equal. These two selected wavelengths were employed to determine the concentration of ALK from the mixture of ALK and AML (Figure 1). The difference in absorbance at these two wavelengths ($A_{256.0} - A_{354.5}$) cancels out the contribution of absorbance of AML in mixture.

Validation of proposed method [18]

The proposed method has been validated for the simultaneous determination of ALK and AML in tablet dosage form using following parameters.

Linearity

Linear correlation was obtained between absorbance Vs concentrations of ALK in concentration range 20-120 µg/Ml and AML in concentration range of 10-60 µg/Ml for proposed method. Regression parameters are mentioned in table 1

Accuracy The recovery experiment was performed by the standard addition method. The mean recoveries were 99.87 ± 1.25 and 99.9. ± 1.38 % for ALK and AML, respectively (Table 1). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 2

Method precision (% Repeatability)

The % RSD values for ALK and AML were found to be 1.94 and 1.61 for Absorbance correction method (Table 1 & Table 3). The low values of RSD indicate the proposed methods are repeatable.

Intermediate precision

The low RSD values of interday (1.45-2.01% and0.73-1.84%) and intraday (1.07-1.96 % and 0.47-1.72 %) variations for ALK and AML, respectively, reveal that the proposed method is precise (Table 1).

TABLE 1 Regression analysis data and summary of validation parameters for the proposed method.

PARAMETERS	ABSORBANCE CORRECTION METHOD	
	ALK	AML
Concentration range (µg/mL)	20-120	10-60
Slope	0.001	0.012
Intercept	0.020	0.076
Correlation coefficient	0.999	0.997
LOD (µg/mL)	3.68	0.74
LOQ (µg/mL)	11.16	2.24
% recovery (Accuracy, n = 6)	99.87 ± 1.25	99.9. ± 1.38
Repeatability (% RSD, n = 6)	1.94	1.61
Interday (n = 6)	1.45-2.01	0.73-1.84
Intraday (n = 6)	1.07-1.96	0.47-1.72

TABLE 2 Recovery Data for the Proposed Method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean % Recovery ± SD*
ALK	I	40	50 %	99.11 ± 0.75
	II	40	100 %	100.2 ± 1.39
	III	40	150 %	100.3 ± 1.6

AML	I	20	50 %	99.12 ± 0.91
	II	20	100 %	100.8 ± 1.79
	III	20	150 %	99.77 ± 1.45

* Mean % Recovery ± SD of six observations.

TABLE 3 Precision Data for Aliskiren and Amlodipine

ALK and AML (40 µg/ml)	**Absorbance diffrence**	**Absorbance at 354.5nm**
1	0.043	0.399
2	0.042	0.394
3	0.041	0.391
4	0.041	0.389
5	0.042	0.385
6	0.043	0.402
Mean	0.042	0.393
S.D.	0.00081	0.00634
%CV	1.94	1.61

TABLE 4 Analysis of Marketed Formulation of Aliskiren and Amlodipine by Proposed Method (n = 6)

Sample No.	**Label Claim**		**Amount Found**		**% Label Claim**	
	ALK (mg/tab)	AML (mg/tab)	ALK (mg/tab)	AML (mg/tab)	ALK (mg/tab)	AML (mg/tab)
1	150	5	151.7	5.03	101.13	100.7
2	150	5	151.6	4.96	101.09	99.2
3	150	5	149.7	5.08	99.8	101.6
4	150	5	149.1	5.07	99.4	101.4
5	150	5	153.0	4.92	102	98.4
6	150	5	149.1	5.02	99.4	100.4

Mean	150.70	5.01	100.47	100.28
S.D.	1.62	0.06	1.08	1.23

LOD and LOQ

LOD for ALK and AML were found to be 3.68 µg/Ml and 0.74 µg/Ml, respectively. LOQ for ALK and AML were found to be 11.16 µg/Ml and 2.24 µg/Ml, respectively (Table 1). These data show that method is sensitive for the determination of ALK and AML.

Assay of the pharmaceutical formulation

The proposed validated method was successfully applied to determine ALK and AML in their combined dosage form. The spectrum of sample is shown in Fig. 2. The results obtained for ALK and AML were comparable with the corresponding labeled amounts (Table 4).

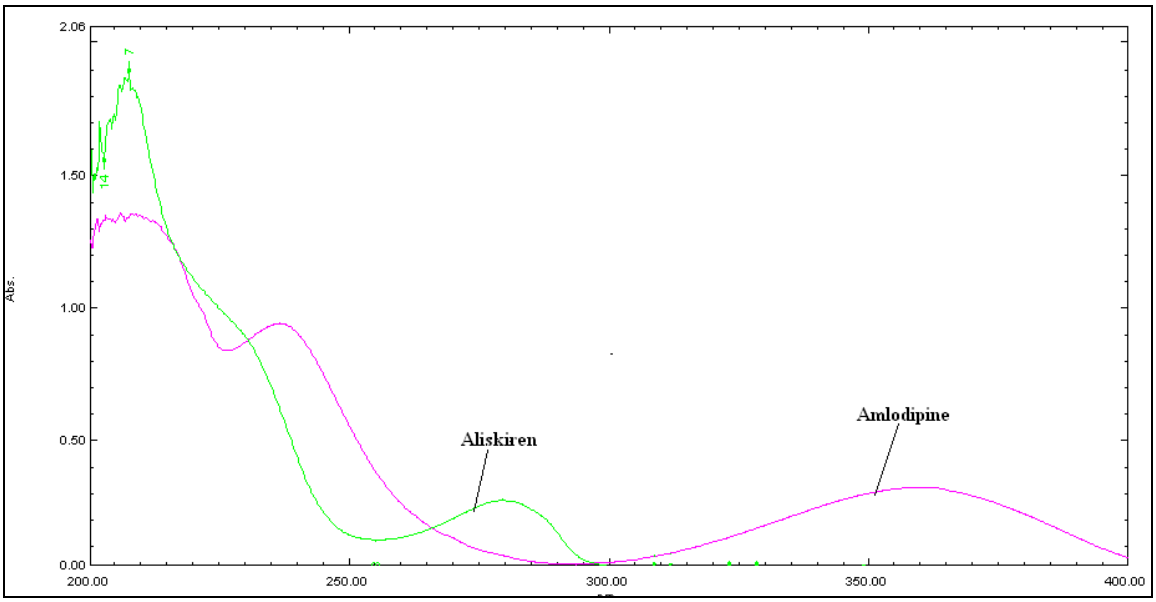

FIGURE: 1 Overlain absorption spectra of standard solution of Aliskiren (60 ug /ml) and Amlodipine (30ug/ml) in methanol

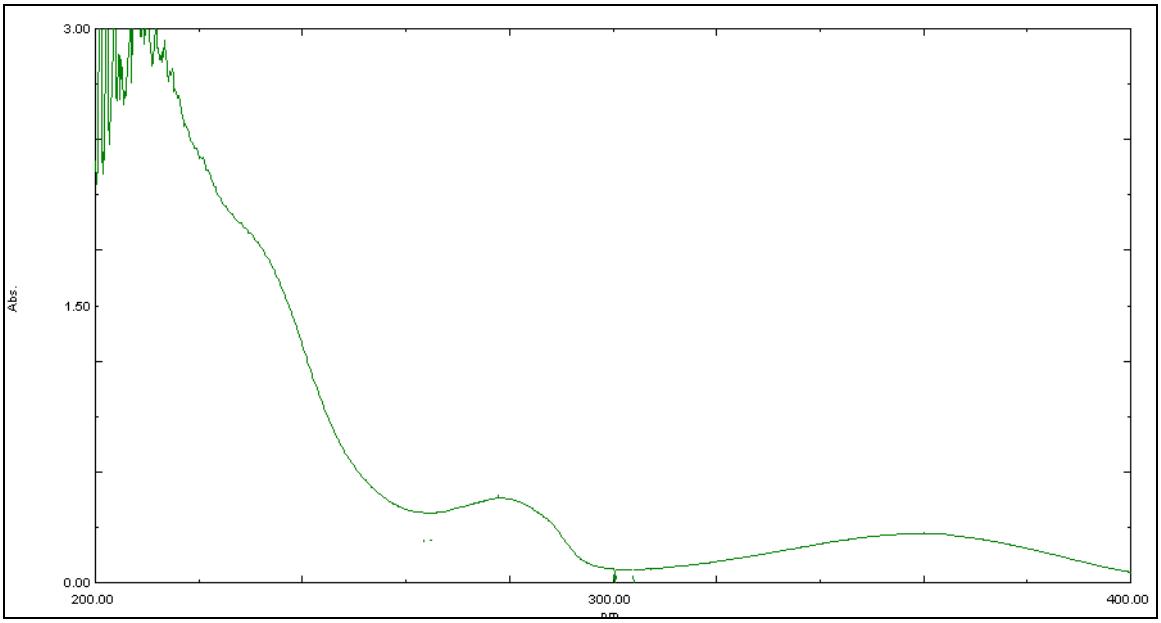

FIGURE 2 Absorption Spectra of sample solution of Aliskiren and Amlodipine in methanol

CONCLUSION:

In this proposed method the linearity is observed in the concentration range of 20 – 120 µg/Ml with co-efficient of correlation, (r^2) = 0.999 and 10 – 60 µg/Ml with co-efficient of correlation (r^2) = 0.997 for ALK and AML, respectively.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the ALK and AML in combined dosage form without any interference of the excipients.

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